

Biotechnology applications in food processing: Can developing countries benefit?

1. Introduction

Biotechnology includes a wide range of diverse technologies and they may be applied in each of the different food and agriculture sectors. It includes technologies such as gene modification (manipulation) and transfer; the use of molecular markers; development of recombinant vaccines and DNA-based methods of disease characterisation/diagnosis; in-vitro vegetative propagation of plants; embryo transfer and other reproductive technologies in animals or triploidisation in fish. It also includes a range of technologies used to process the raw food materials produced by the crop, fishery and livestock sectors. This is the area that will be considered in this conference, the 11th one to be hosted by the [FAO Biotechnology Forum](#) since it was launched in March 2000. It is an area that receives relatively little attention from the media, but which is very important for food security in many developing countries.

Biotechnology in the food processing sector targets the selection and improvement of microorganisms with the objectives of improving process control, yields and efficiency as well as the quality, safety and consistency of bioprocessed products. Microorganisms or microbes are generic terms for a group of living organisms which are microscopic in size, and include bacteria, yeasts and moulds.

Fermentation is the process of bioconversion of organic substances by microorganisms and/or enzymes (complex proteins) of microbial, plant or animal origin. It is one of the oldest forms of food preservation which is applied globally. Indigenous fermented foods such as bread, cheese and wine, have been prepared and consumed for thousands of years and are strongly linked to culture and tradition, especially in rural households and village communities. It is estimated that fermented foods contribute to about one-third of the diet worldwide.

During fermentation processes, microbial growth and metabolism (the biochemical processes whereby complex substances and food are broken down into simple substances) result in the production of a diversity of metabolites (products of the metabolism of these complex substances). These metabolites include enzymes which are capable of breaking down carbohydrates, proteins and lipids present within the substrate and/or fermentation medium; vitamins; antimicrobial compounds (e.g. bacteriocins and lysozyme); texture-forming agents (e.g. xanthan gum); amino acids; organic acids (e.g. citric acid, lactic acid) and flavour compounds (e.g. esters and aldehydes). Many of these microbial metabolites (e.g. flavour compounds, amino acids, organic acids, enzymes, xanthan gums, alcohol etc.) are produced at the industrial level in both developed and developing countries for use in food processing applications. A considerable volume of current research both in academia and industry targets the application of microbial biotechnology to improve the production, quality and yields of these metabolites.

Fermentation is globally applied in the preservation of a range of raw agricultural materials (cereals, roots, tubers, fruit and vegetables, milk, meat, fish etc.). Commercially produced fermented foods which are marketed globally include dairy products (cheese, yogurt, fermented milks), sausages and soy sauce. Certain microorganisms associated with fermented foods, in particular strains of the *Lactobacillus* species, are probiotic i.e. used as live microbial dietary supplements or food ingredients that have a beneficial effect on the host by influencing the composition and/or metabolic activity of the flora of the gastrointestinal tract. Probiotic bacterial strains are also produced and commercially marketed in many developed countries.

In developing countries, fermented foods are produced primarily at the household and village level, where they find wide consumer acceptance. Food fermentations contribute substantially to food safety and food security, particularly in the rural areas of many developing countries. Traditional fermentation processes used in the production of these foods are uncontrolled and are dependent on microorganisms from the environment or the fermentation substrate for initiation of the fermentation processes. Such processes, therefore, result in products of low yield and variable quality. Microorganisms and metabolic pathways associated with the production of fermented foods are the subject of considerable research, targeting strain isolation and identification; improvement of the efficiency of fermentation processes and the quality, safety and consistency of fermented foods. Much of this research incorporates the use of genetic technologies for strain development and improvement, and for diagnostic studies.

While microorganisms are beneficial in most fermentation processes, some may pose the risk of food contamination and can cause food-borne illness. Diagnostic methodologies which integrate the use of molecular genetic techniques, enhance the speed and sensitivity of microbial testing and are increasingly being applied in developing countries.

In conferences hosted by the FAO Biotechnology Forum, clearly defined topics of relevance to agricultural biotechnology in developing countries are discussed for a limited amount of time. The topic here is the **application of biotechnology to the processing of food (including beverages) produced from agriculture**. This e-mail conference discusses biotechnological tools and options that are applicable to the study and improvement of microorganisms which offer potential for improving the quality, safety and consistency of fermented foods; improving efficiency in the production of fermented foods, food ingredients, food additives and food processing aids (enzymes); diversifying the outputs of fermentation processes and, finally, improving diagnostic and identification systems applicable to foods. Applications of biotechnology to plants or animals to improve their food processing properties (e.g. development of the Flavr Savr tomato variety, genetically modified to reduce its ripening rate) or to produce proteins from genetically modified (GM) microorganisms to improve plant or animal production (e.g. production of bovine somatotropin (BST), a hormone increasing milk production in dairy cows, by GM bacteria) are not considered here. Finally, the conference topic covers applications of biotechnology to processing of food and not to processing of non-food agricultural products (e.g. timber) or to applying biotechnology to microorganisms for environmental purposes (bioremediation, biofuels etc.).

2. Current Status of Biotechnology in Food Processing

2.1 Biotechnology in food fermentation

Microorganisms are an integral part of the processing system during the production of fermented foods. Microbial cultures can be genetically improved using both traditional and molecular approaches, and improvement of bacteria, yeasts and moulds is the subject of much academic and industrial research. Traits which have been considered for commercial food applications in both developed and developing countries include sensory quality (flavour, aroma, visual appearance, texture and consistency), virus (bacteriophage) resistance in the case of dairy fermentations, and the ability to produce antimicrobial compounds (e.g. bacteriocins, hydrogen peroxide) for the inhibition of undesirable microorganisms. In many developing countries, the focus is on the degradation or inactivation of natural toxins (e.g. cyanogenic glucosides in cassava), mycotoxins (in cereal fermentations) and anti-nutritional factors (e.g. phytates).

2.1.1 Traditional approaches

Traditional methods of genetic improvement such as classical mutagenesis and conjugation have been the basis of industrial starter culture development in bacteria (a culture used to start a food fermentation is known as a starter culture), while hybridisation has been used in the improvement of yeast strains which are widely applied industrially in baking and brewing applications.

a) Classical mutagenesis

This involves the production of mutants by the exposure of microbial strains to mutagenic chemicals or ultraviolet rays to induce changes in their genomes. Improved strains thus produced are selected on the basis of specific properties such as improved flavour-producing ability or resistance to bacterial viruses. Such mutants may, however, show undesirable secondary mutations which can influence the behaviour of cultures during fermentation.

b) Conjugation

This is a natural process whereby genetic material is transferred among closely related microbial species as a result of physical contact between the donor and the recipient microorganism. Conjugational gene exchange allows both plasmid-localised and chromosomal gene transfer (a plasmid is a circular self-replicating non-chromosomal DNA molecule found in many bacteria, capable of transfer between bacterial cells of the same species, and occasionally of different species).

c) Hybridisation (i.e. sexual breeding or mating)

Sexual reproduction in yeasts, and thus genetic recombination, has led to improvements in yeasts. For example, crossing of haploid yeast strains with excellent gassing properties and with good drying properties could yield a novel strain with both good gassing and drying properties.

2.1.2 Molecular approaches

a) Genetic modification

Recombinant DNA approaches have been used for genetic modification of bacterial, yeast and mould strains to promote expression of desirable genes, to hinder the expression of others, to alter specific genes or to inactivate genes so as to block specific pathways. The successful application of genetic modification for food bioprocessing applications requires the development and use of food grade vectors, i.e. plasmids which do not contain antibiotic resistance genes as markers and which consist of DNA sequences from microorganisms which are generally recognised as safe (GRAS).

GM yeasts appropriate for brewing and baking applications have been approved for use (e.g. approval was granted in the United Kingdom for use of a GM yeast (*Saccharomyces cerevisiae*) in beer production, containing a transferred gene from the closely related *Saccharomyces diastolicus*, allowing it to better utilise the carbohydrate present in conventional feedstocks). None of these GM yeasts are, however, used commercially.

b) Genetic characterisation

The genetic characterisation of microbial strains through the use of molecular diagnostic techniques can contribute tremendously to the understanding of fermentation processes. Molecular diagnostics provide outstanding tools for the detection, identification and characterisation of microbial strains for bioprocessing applications and for the improvement of fermentation processes. The application of these and other related techniques, along with the development of molecular markers for bacterial strains, greatly facilitates understanding of the ecological interactions of microbial strains, their roles, succession, competition and prevalence in food fermentations and allows the correlation of these features to desirable quality attributes of the final product.

c) Genomics

In recent years, the genome sequences of many food-related microorganisms have been completed (e.g. *Saccharomyces cerevisiae*, commonly known as baker's or brewer's yeast, was the first eucaryote to have its genome sequenced - in 1996) and large numbers of microbial genome sequencing projects are also underway (see e.g. <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Genome> for an update). Functional genomics, a relatively new area of research, aims to determine patterns of gene expression and interaction in the genome, based on the knowledge of extensive or complete genomic sequence of an organism. It can provide an understanding of how microorganisms respond to environmental influences at the genetic level (i.e. by expressing specific genes) in different situations or ecologies, and should therefore allow adaptation of conditions to improve technological processes. For a range of microorganisms, it is now possible to observe the expression of many genes simultaneously, even those with unknown biological functions, as they are switched on and off during normal development or while an organism attempts to cope with pathogens or changing environmental conditions. For example, a recent paper by Cooper and colleagues (2003, available at <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=298728>) describes their use of DNA microarrays to analyse expression of all 4,290 genes of the model bacterium *Escherichia coli* after 20,000 generations of evolution in a glucose-limited medium. Functional genomics can, for example, shed light on common genetic mechanisms which enable microorganisms to use certain sugars during fermentation, as well as on genetic differences allowing some strains to perform better than others. It holds great potential for defining and modifying elusive metabolic mechanisms used by microorganisms. Moving from the gene to the protein level, it should also be mentioned that proteomics, an approach aiming to identify and characterise complete sets of protein, and protein-protein interactions in a given species, is also a very active area of research which offers potential for improving fermentation technologies.

2.2 Biotechnology in the production of enzymes

Enzymes are biological catalysts used to facilitate and speed up metabolic reactions in living organisms. They are proteins and require a specific substrate on which to work. Their catalysing conditions are set within narrow limits, e.g. optimum temperature, pH conditions and oxygen concentration. Most enzymes are denatured at temperatures above 42°C. However, certain bacterial enzymes are tolerant to a broader temperature range. Enzymes are essential in the metabolism of all living organisms and are widely applied as processing aids in the food and beverage industry.

In the past, enzymes were isolated primarily from plant and animal sources, and thus a relatively limited number of enzymes were available to the food processor at a high cost. Today, bacteria and fungi are exploited and used for the commercial production of a diversity of enzymes. Several strains of microorganisms have been selected or genetically modified to increase the efficiency with which they produce enzymes. In most cases, the modified genes are of microbial origin, although they may also come from different kingdoms. For example, the DNA coding for chymosin, an enzyme found in the stomach of calves, that causes milk to curdle during the production of cheese, has been successfully cloned into yeasts (*Kluyveromyces lactis*), bacteria (*Escherichia coli*) and moulds (*Aspergillus niger* var. *awamori*). Chymosin produced by these recombinant microorganisms is currently commercially produced and is widely used in cheese manufacture.

The industrial production of enzymes from microorganisms involves culturing the microorganisms in huge tanks where enzymes are secreted into the fermentation medium as metabolites of microbial activity. Enzymes thus produced are extracted, purified and used as processing aids in the food industry and for other applications. Purified enzymes are cell free entities and do not contain any other macromolecules such as DNA.

Genetic technologies have not only improved the efficiency with which enzymes can be produced, but they have increased their availability, reduced their cost and improved their quality. This has had the beneficial impact of increasing efficiency and streamlining processes which employ the use of enzymes as processing aids in the food industry.

In addition, through protein engineering, it is possible to generate novel enzymes with modified structures that confer novel desired properties, such as improved activity or thermostability or the ability to work on a new substrate or at a higher pH. Directed evolution is one of the main methods currently used for protein engineering. This technique involves creating large numbers of new enzyme variants by random genetic mutation and subsequently screening them to identify the improved variants. This process is carried out repeatedly, thus mimicking natural evolution processes.

2.3 Biotechnology in the production of food ingredients

As described in the Introduction, flavouring agents, organic acids, food additives and amino acids are all metabolites of microorganisms during fermentation processes. Microbial fermentation processes are therefore commercially exploited for production of these food ingredients. Metabolic engineering, a new approach involving the targeted and purposeful manipulation of the metabolic pathways of an organism, is being widely researched to improve the quality and yields of these food ingredients. It typically involves alteration of cellular activities by the manipulation of the enzymatic, transport and regulatory functions of the cell using recombinant DNA and other genetic techniques. Understanding the metabolic pathways associated with these fermentation processes, and the ability to redirect metabolic pathways, can increase production of these metabolites and lead to production of novel metabolites and a diversified product base.

2.4 Biotechnology in diagnostics for food testing

Many of the classical food microbiological methods used in the past were culture-based, with microorganisms grown on agar plates and detected through biochemical identification. These methods are often tedious, labour-intensive and slow. Genetic based diagnostic and identification systems can greatly enhance the specificity, sensitivity and speed of microbial testing. Molecular typing methodologies, commonly involving the polymerase chain reaction (PCR), ribotyping (a method to determine fragment lengths and differences between bacteria at the species or sub-species (strain) level, using restriction enzyme digests of pulsed-field gel electrophoresis (PFGE) analysis of ribosomal ribonucleic acids (rRNA) genes) and pulsed-field gel electrophoresis (PFGE, a method of separating large DNA molecules that can be used for typing microbial strains), can be used to characterise and monitor the presence of spoilage flora (microbes causing food to become unfit for eating), normal flora and microflora in foods. Random amplified polymorphic DNA (RAPD) or amplified fragment length polymorphism (AFLP) molecular marker systems can also be used for the comparison of genetic differences between species, subspecies and strains, depending on the reaction conditions used. The use of combinations of these technologies and other genetic tests allows the characterisation and identification of organisms at the genus, species, sub-species and even strain levels, thereby making it possible to pinpoint sources of food contamination, to trace microorganisms throughout the food chain or to identify the causal agents of foodborne illnesses. Monoclonal and polyclonal antibodies can also be used for diagnostics, e.g. in enzyme-linked immunosorbent assay (ELISA) kits.

Microarrays are biosensors which consist of large numbers of parallel hybrid receptors (DNA, proteins, oligonucleotides). Microarrays are also referred to as biochip, DNA chip, DNA microarray or gene arrays and offer unprecedented opportunities and approaches to diagnostic, DNA chip, and detection methods. They can be used for the detection of pathogens, pesticides and toxins and offer considerable potential for facilitating process control, the control of fermentation processes and monitoring the quality and safety of raw materials.

3. Some Issues Relevant to Developing Countries

This conference deals with the application of biotechnology to food processing in developing countries. Biotechnological research as applied to bioprocessing in the majority of developing countries, targets development and improvement of traditional fermentation processes. In this section, we consider some areas specifically relevant to developing countries and list some key issues that should be considered by participants in this conference.

3.1 Socio-economic and cultural factors

Traditional fermentation processes employed in most developing countries are low input, appropriate food processing technologies with minimal investment requirements. They make use of locally produced raw materials and are an integral part of village life. These processes are, however, often uncontrolled, unhygienic and inefficient and generally result in products of variable quality and short shelf lives. Fermented foods, nevertheless, find wide consumer acceptance in developing countries and contribute substantially to food security and nutrition.

- How will applications of biotechnology to fermented foods impact on these socio-economic and cultural factors?

3.2 Infrastructural and logistical factors

Physical infrastructural requirements for the manufacture, distribution and storage (e.g. by refrigeration) of microbial cultures or enzymes on a continuous basis is generally available in urban areas of many developing countries. However, this is not the case in most rural areas of developing countries.

- Should research be undertaken to ensure that individuals at all levels can benefit from applications of biotechnology in food fermentation processes, i.e. should logistical arrangements for starter culture development be integrated into biotechnological research targeting improvement of traditional fermentations? What is required for the level of fermentation technologies and process controls to be upgraded in order to increase efficiency, yields and the quality and safety of fermented foods in developing countries?

3.3 Nutrition and food safety

Fermentation processes enhance the nutritional value of foods through the biosynthesis of vitamins, essential amino acids and proteins, through improving protein and fibre digestibility; enhancing micronutrient bioavailability and degrading antinutritional factors. Many bacteria in fermented foods also exhibit functional properties (probiotics).

The safety of fermented food products is enhanced through reduction of toxic compounds, such as mycotoxins and cyanogenic glucosides, and production of antimicrobial factors, such as bacteriocins, carbon dioxide, hydrogen peroxide and ethanol, which facilitate inhibition or elimination of food-borne pathogens.

- Are the nutritional characteristics (and safety aspects) of fermented foods adequately documented and appreciated in developing countries? Is there a need for consumer education about the benefits of fermented foods?

3.4 Intellectual property rights (IPRs)

The processes used in the more advanced areas of agricultural biotechnology tend to be covered by IPRs and these rights tend to be owned by parties in developed countries. This applies also to biotechnological processes used in food processing. On the other hand, many of the traditional fermentation processes applied in developing countries are based on traditional knowledge.

In addition to biotechnology processes, microbial strains may also be the object of IPRs. For example, an era of massive private investment in biotechnology was initiated when the United States Supreme Court ruled in 1980 (in the *Diamond versus Chakrabarty* case) that a live GM bacterium (of the genus *Pseudomonas*, modified to degrade components of crude oil) could be patented. Many of the microorganisms associated with traditional fermentation processes in developing countries are unique. Issues of ownership will become increasingly important as bacterial strains are characterised and starter cultures are developed in developing countries.

- How should food scientists, researchers, industry and governments in developing countries approach these issues?

- A considerable volume of research into the development and improvement of fermentation processes is currently taking place worldwide. Are the research results from developing countries adequately documented? Who owns this information? Are cell banks being developed to protect microbial strains characterised in developing countries?

3.5 Commercial opportunities

Biotechnological innovations have greatly assisted in industrialising production of certain indigenous fermented foods. Indonesian tempe and Oriental soy sauce are well known examples of indigenous fermented foods that have been industrialised and marketed globally. The results of biotechnology research will lead to fermented foods of improved quality, safety and consistency.

- Should biotechnology developments in developing countries target commercialisation? Should they target diversification into new value-added products? Should biotechnology development be linked to technological developments in food processing?

- Can the application of biotechnology to food processing allow farmers in developing countries to add value to their agricultural products (for export or for local consumption) and improve their revenues?

3.6 Appropriateness of food processing biotechnology in developing countries

As with any commitment of resources, investments in biotechnology for food processing should be weighed up against other potential uses of these resources in developing countries.

- How relevant and worthwhile can such investments be for developing countries?

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